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Plant selection for rain gardens: response to simulated cyclical flooding of 15 perennial species

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Abstract: Plant selection for rain gardens can be complicated, as cyclic flooding and a gradient of moisture level are expected in the depression structure of a rain garden. However, few studies to date have quantified how plant establishment is affected by rain garden moisture dynamics. This study investigated tolerance of 15 candidate perennial species, which experienced flooding cycles consisting of 1-day and 4-day inundation and draining phases. In this study, detection of species suitability using survival and growth measurements coupled with the stress indicator (i.e. chlorophyll fluorescence) provided a valid framework for wider use in plant selection for rain gardens. The methodology is also confident in predicting the possible placing in different plant moisture zones. All species survived the cyclic flooding treatments and grew to their maximum. Photosynthesis and physical growth in only a few candidate species (e.g. *Amsonia tabernaemontana var. salicifolia, Gaura lindheimeri, Sanguisorba tenuifolia 'Purpurea'* and *Thalictrum aquilegifolium*) tended to be inhibited by treatments adopting 4-day cyclic flooding, whilst tolerance to 1-day cyclic flooding was clearly demonstrated in most species. Analysis suggests that most species assumed to withstand infrequent to periodic inundation, such as *Iris sibirica*, *Filipendula purpurea* and *Miscanthus sinensis*, are resilient species and are sensible for use in a wider range of rain garden moisture conditions from damp depression bottom to dry margin. Species assumed to be intolerant of inundation such as *Gaura lindheimeri* may be successful in the rain garden environment, but they are recommended for the dryer zones.

Keywords: Rain garden; Cyclic flooding; Perennial; Plant selection; Adaptation

1 Introduction

Rain gardens are planted depressions which rely on vegetation and soils to mitigate excess runoff accommodated from builidings, pavements and roads [1]. Such features are often adopted in the public right-of-way, adding aesthetic value and biodiversity into areas that would otherwise be devoid of vegetation [2]. Mixes of perennials (particularly flowering forbs and ornamental grasses) currently receive considerable attention as alternative vegetation options. Such mixes may be cost-effective and multi-functional: enhancing stormwater infiltration and evaporation, promoting visual aesthetics (i.e. variation in forms, flower colours, blooming periods and foliage textures), encouraging biodiversity, as well as being suitable for use on sites at any scale [3, 4].

1.1 Rain garden moisture dynamics

Rain gardens rely on natural rainfall as their source of irrigation, and are normally specified to dewater within a period from 24 hours to a maximum saturated period of 96 hours [5, 6]. Therefore, rain gardens will undergo cyclical change, from periodic waterlogging through to dryer conditions. Vertical and horizontal moisture gradients also develop: a typical rain garden can be characterised as having three moisture zones, including an often mosit to waterlogged depression bottom, an occasionally flooded side-slope having a moderate moisture status, and a dryish upland margin [7] (Fig. 1).

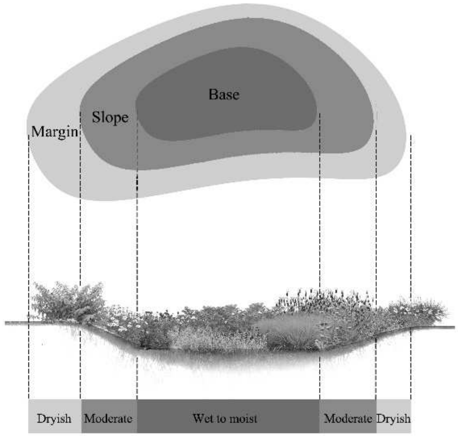

Fig. 1. Illustration of the rain garden moisture gradient

1.2 Plant selection for rain gardens

Cyclic flooding leads to conditions in rain gardens that are similar to a transition zone between a terrestrial system and a wetland, with a frequent switching between flooding and draining, with the added complication of the interaction with the gradient of moisture levels throughout the 'margin-slope-bottom' depression structure. Since perennial species have a remarkable diversity in tolerance to flooding conditions, typifying suitable vegetation types and plants for rain garden application is never a simple task. Inappropriate species adoption in the implementation of rain gardens can result in the failure of planting, which may lead to unnatural and sometimes unpleasing visual effects. There are evidences of increased infiltration totals and rates in rain garden arising from preferential flow pathways provided by plants [8], as well as the improved soil permeability and porosity as a result of enlarged and elongated soil pores following vegetation root turnover [9, 10]. Therefore, the loss of vegetation due to failure of planting in a rain garden could result in a considerable reduction its contribution to stormwater infiltration though the subsoil characters often play a major role in stormwater runoff treatment performance.

It is important to make planting suggestions on the basis of plant responses and adaptations to rain garden moisture dynamics. However, current technical manuals and scientific research show remarkably little evidence to fully reflect as to how cyclic flooding and moisture gradient may have influenced the growth of plants preferred by professionals (herbaceous species in particular). For instance, Vander Veen [11] monitored the vegetative health of a series of North American native forbs and grasses in retention basins allowing natural precipitation and infiltration. This study visually judged plant growth conditions on saturated days, as well as measured the maximum number of consecutive days a plant species might tolerate saturated or dry soil till visible damages were found. However, this methodology is not easily replicated in practice, and did not take account of the typical cyclical flooding of a rain garden

Dylewski *et al*. [12] soaked potted plants in a water bath for 3 days and 7 days and took them out to allow one week of draining without irrigation until the next flood cycle began. The soaking and draining phases were repeated to create different cyclical flooding periods. A non-flooded group with regular irrigation to maintain the substrate at a moisture level of between 0.20 and 0.25 $m^3 \cdot m^{-3}$ (i.e. the absolute value of soil water content) was adopted as the control group. The study used three standard landscape shrubs: (*Ilex glabra* 'Shamrock', *Itea virginica* 'Henry's Garnet' and *Viburnum nudum* 'Winterthur'). Survival rates and growth characteristics such as shoot dry weight, root dry weight and growth index (i.e. [(height + widest width + width perpendicular to widest width) ÷ 3]) were used as indicator factors for determining the effect of cyclic flooding on the planting developments. Elevated mortality rate was detected in all the three shrub species, whilst the growth characteristics in all three species were significantly reduced because of the cyclic flooding treatments. However, Dylewski *et al*. concluded that all species were tolerant of cyclic flooding.

Some tolerant species may show a low O_2 quiescence strategy that reduces the use of carbohydrates and energy or conserves growth upon submergence to prolong survival, whilst some genotypes may elongate shoots that emerge out of submergence to restore gas exchange [13, 14]. Therefore, species' suitability cannot be determined solely depending on their physiological growths. Waterlogging stresses either directly or indirectly decrease the leaf photosynthetic efficiency and cause photoinhibition (i.e. the light-induced reduction in the photosynthetic capacity of a plant) prior to visible deteriorations in plants [15, 16]. Photoinhibition can be detected from the reduction in the yield of chlorophyll fluorescence [17]. A few studies have adopted leaf chlorophyll fluorescence as an effective indicator to evaluate waterlogging stress in amenity plants, and this method provides more insights on predicting the further developments of the candidate species in expected soil moisture profile [18-20]. However, the use of chlorophyll fluorescence for evaluating tolerance in the candidate plants under the stress of typical cyclic flooding in rain garden remains unreported. A reliable and simple methodological approach is therefore needed that can be used to predict the suitability of potential species for rain gardens, and their possible placing in different plant moisture zones.

1.3 Objectives

Many of the established rain garden plant lists are not based on data from replicated experiments, and there has been little research that evaluates the interaction between specific plants and the dynamic spatiotemporal moisture distribution in rain gardens. This leaves a major research gap in expanding plant options for rain gardens. This study focuses on quantitatively understanding the effects of cyclic flooding on the establishment of a series of candidate perennial species. This paper aims to provide insight into developing a framework and methodology for selecting suitable perennial species for rain garden hydrology dynamics, which can be useful for designers who make planting decisions.

2 Methods

The experiment enabled observation of the response of 15 candidate perennials to rain garden moisture dynamics by following the 'pot-in-pot' methodology of Dylewski *et al*. [12] using periodic water bath and draining to simulate the cyclic flooding. In addition, stress in candidate plants was detected by evaluating the measurements of leaf chlorophyll fluorescence.

2.1 Site and materials

The study was conducted in an unheated, ventilated greenhouse situated at Norton Nursery, Sheffield, UK (1°27'44.9"W, 53°20'00.6"N). Over the course of the experiment a minimum temperature of 7.6°C was recorded and a maximum air temperature of 34.3°C, while the daily relative humidity varied between 15.0 % and 89.8%. The artificial substrate was a mix of sharp sand and sterilised topsoil and peat at a volume ratio of 5:2:3, which was classified as a gritty sandy loam (67.2% sand, 13.7% silt and 0.01% clay) with an organic matter content of 8.21% in volume and a pH of 7.9. The growing medium was free-draining with a porosity of 66.5% and a permeability of 5.7 cm/hour. The substrate not only enables effective drainage, but also has sufficient organic components to retain soil water and sustain nutrients for supporting vegetation development. Similar media mixes are widely adopted in technical guidance for rain gardens, such as Woelfle-Erskine & Uncapher [6] and Prince George's County [21].

The candidate speices consisted of eleven forbs and four grasses: *Amsonia tabernaemontana var. salicifolia*, *Astilbe* 'Purple Lance', *Calamagrostis brachytricha*, *Caltha palustris*, *Deschampsia flexuosa*, *Filipendula purpurea*, *Gaura lindheimeri*, *Hemerocallis* 'Golden Chimes', *Iris sibirica*, *Miscanthus sinensis*, *Molinia caerulea*, *Rudbeckia fulgida var. deamii*, *Sanguisorba tenuifolia* 'Purpurea', *Thalictrum aquilegifolium*, *Veronicastrum virginicum*. Most of these species were selected from genera that are widely recommended in rain garden guidance [2], and were identified as being capable to acclimate to wetter/dryer periods according to botanic documents [7, 22, 23]. However, within this a range of species with different potential tolerances were selected. For example, *Gaura lindheimeri* is typical of dryer sites, whist Caltha palustris is restricted in the wild to permanently moist sites. Plants were supplied in 9cm diameter pots from Orchard Dene Nurseries (Oxfordshire, UK). A single plant of each species was planted into one 2L freely drained pot with drainage holes on 15 April 2013. There were 15 pots for each species, which were then watered every other day to maintain substrate moisture for a month to establish prior to treatments.

2.2 Simulation of cyclic flooding

Five single pot replicates of individual species were given each of the experimental treatments. The cyclic flooding treatments commenced on 28 May 2013. Treatments consisted of:

1. **Non-flooded control group** in which plants were carefully irrigated to maintain their substrate moisture between 20% and 25% following the instructions of Bailey [24] and Dylewski et al. [12] to keep the plants well watered in a mesic substrate. The volumetric substrate moisture at 50 mm depth per pot was measured daily between 9 am and 10 am throughout the entire experiment using a handheld HH2 meter and SM200 moisture sensor (Delta-T, Cambridge, United Kingdom). Soil moisture was obtained for three measurements per substrate each time and calculated the mean value.

2. **1d group** that was flooded to substrate level for a one-day (24 hours) short interval cyclic flooding.

3. **4d group** that was flooded to substrate level for a four-day (96 hours) longer-term interval cyclic flooding.

Plants in 1d and 4d group were flooded to the level of the substrate by being placed in saturated water tanks to simulate flooding conditions in rain garden profile (Fig. 2). Polyethylene water tanks were open top with a surface area of 1000 mm by 500 mm (1.5 m^2) and a depth of 400 mm. Plants in 1d and 4d group were taken out for a 4-day draining after each 1-day or 4-day inundation until the next flooding cycle was repeated. During the 4-day draining periods, pots were placed on flat concrete paving at 200 mm spacing, whilst no irrigation was applied until the onset of the next flood cycle. During the flooding treatments in 4d group, water was added into the water tanks every day to maintain the water table at the level of the substrate. Plants from the control group were placed on flat ground at 200 mm spacing.

Fig. 2. Plants in the water bath to simulate conditions in a typical flooding cycle (Photo was taken by the first author, May 2013).

The main focus of this study was to evaluate the growth and survival rather than the further development of candidate species under the effect of a typical flooding cycle in rain gardens. During the study, indoor temperature was high at times, so that all the herbaceous plants tended to grow very fast to maximum (i.e. no visible growing tissues observed for at least one to two weeks). All experimental treatments were concluded on 28 June 2013 (32 days in total). Plants in the 1d and 4d treatments experienced a total of seven and four flooding cycles, respectively.

2.3 Growth and survival of plants

Survival rate, as well as height and spread of individual plants were measured at experiment termination. In this study, plant height was determined from the bottom to the highest leaf apex. Each plant was measured from above to determine the plant length and width, and then the mean value was calculated to assess spread. Plant samples were destructively harvested immediately afterwards the heights and spreads were obtained. Shoots were removed from the root ball and dried at 80˚C for 48h to measure the shoot dry weight (SDW). Roots were gently hand-washed free of substrate in tap water, immersed in tap water overnight, rinsed three times with distilled water, and then dried similarly to measure the root dry weight (RDW).

2.4 Stress detection via leaf chlorophyll fluorescence

Leaf chlorophyll fluorescence was determined individually by species to evaluate the flooding-induced stress in candidate plants. F_v/F_m ratio as one of the most used chlorophyll fluorescence parameters was adopted in this study. F_v is defined as the difference between the measurements of the maximum level and minimum level of fluorescence yield, and F_m refers to the maximum level of fluorescence yield [17, 25]. Chlorophyll fluorescence were measured by attaching light exclusion clips to the leaf and allowing leaves to be dark-adapted for 30 min, and fluorescence values were obtained using a Handy PEA portable fluorescence spectrometer (Hansatech Instruments, Norfolk, UK). Three leaves were randomly selected for measurements per plant to calculate the mean chlorophyll fluorescence value. Each selected leaf was tagged, ensuring that the measurements were taken from the same leaf for the whole duration of this study.

Leaf chlorophyll fluorescence in each plant was measured immediately after each flooding period and before the next flooding cycle started in 1d and 4d groups. Fluorescence values in the control group were obtained at the same time when any of the other two groups were measured.

In this study, plant stress was estimated based on an optimal fluorescence value of 0.7. Numerous studies suggested $F_v/F_m < 0.7$ indicated the initiation of stress resulting in the effect of photoinhibition on photosynthesis, reduced growth and leaf necrosis, whilst higher F_v/F_m values than 0.7 indicated better efficiency of photosynthesis and less plant stress [26-28].

2.5 Data analysis

Descriptive results for the survival and stress tolerances to cyclic flooding in each species are provided based on mortality rate at the termination of experiment and the time series chlorophyll fluorescence. One-way ANOVA is introduced to assess the effects of cyclic flooding on plant growths (e.g. SDW, RDW, height and spread). The datasets were checked using Levene's test for normality and homogeneity. No conclusive evidence that the assumptions necessary for ANOVA were infringed was found, and therefore the analysis was performed with untransformed data.

Results of survival, growths and stress tolerance in individual species are scored for further performance evaluation. Ranking methods for cyclic flooding tolerance in individual species are presented in Table 1. Friedman test is then used to see whether there were significant differences between species using all the ratings and produce a league table based on mean rank. Missing data was replaced by median value of the corresponding variable prior to Friedman test. All statistical analyses in this study were performed using SPSS 20.0.

Table 1. Ranking method for cyclic flooding tolerance in individual species

		Rank	Ranking method
Cyclic flooding	**Survival**	5	No mortality at termination of experiment.
		4	40% > Mortality rate ≥ 20% at termination of experiment.
		3	60% > Mortality rate ≥ 40% at termination of experiment.
		2	80% > Mortality rate ≥ 60% at termination of experiment.
		1	Mortality rate ≥ 80% at termination of experiment.
	Growth data (e.g. SDW, RDW, Ht and Spd)[a]	7	Corresponding data was significantly increased due to cyclic flooding treatment and main effect $P \leq 0.01$.
		6	Significant flooding-induced increase was found, while $0.01 < P \leq 0.05$.
		5	Possible increase, i.e. increase in corresponding data was found in cyclic flooding group, while $0.05 < P \leq 0.20$.
		4	Absolute non-significant effect of cyclic flooding treatments was determined, i.e. $0.20 \leq P$.
		3	Possible decrease, i.e. decrease in corresponding data was found in cyclic flooding group, while $0.05 < P \leq 0.20$.
		2	Significant flooding-induced decrease was found, while $0.01 < P \leq 0.05$.
		1	Corresponding data was significantly decreased due to cyclic flooding treatment and main effect $P \leq 0.01$.
	Chlorophyll fluorescence (F_v/F_m)	5	$F_v/F_m > 0.7$ was consistently found in1d and 4d groups throughout the experimental period.
		4	$F_v/F_m > 0.7$ was consistently found in 1d group throughout the experimental period. However, $F_v/F_m < 0.7$ can be occasionally found in 4d group, but overall performance was fairly satisfied.
		3	$F_v/F_m < 0.7$ can be occasionally found in both 1d and 4d groups, but overall performances were fairly satisfied.
		2	At least 50% of the total measurements from 4d group were < 0.7 throughout the experimental period. However, performance in 1d group was generally satisfied.
		1	At least 50% of the total measurements from both 1d and 4d groups were < 0.7 throughout the experimental period.

a: SDW=shoot dry weight, RDW=root dry weight, Ht=height, Spd=spread

3 Results

All the selected species had 100% survival rate in all the three treatments during the whole study. Mean values of shoot dry weight (SDW), root dry weight (RDW), mean height (Ht), and mean spread (Spd) among candidate species across the three durations of flooding cycle are presented in Table 2. Overall, physiological growths in 8 out of 15 candidate species, including *Calamagrostis brachytrica, Caltha palustris, Deschampsia flexuosa, Filipendula purpurea, Hemerocallis 'Golden Chimes', Iris sibirica, Thalictrum aquilegifolium* and *Veronicastrum virginicum*, were not affected by cyclic flooding compared to the regularly irrigated control group.

Table 2. Mean values of shoot dry weight (SDW), root dry weight (RDW), mean height (Ht), and mean spread (Spd) in each selected species, and the effect of duration of flooding cycle on these parameters. Plants in the 1d and 4d group experienced a total of seven and four flood cycles, respectively.

		Control	Cyclic flooding		*F*-statistic	*P*-value
			1d	4d		
Amsonia tabernaemontana var. salicifolia	SDW (g)	6.51a	6.86a	6.72a	0.096	0.909 (ns)
	RDW (g)	4.91b	5.85b	3.46a	6.444	0.013 (*)
	Ht (cm)	56.36a	60.08a	59.66a	0.578	0.576 (ns)
	Spd (cm)	24.05a	20.60a	21.26a	0.985	0.402 (ns)
Astilbe 'Purple Lance'	SDW (g)	26.72a	30.61a	28.11a	0.603	0.574 (ns)
	RDW (g)	48.87a	53.17a	56.28a	0.405	0.683 (ns)
	Ht (cm)	40.18a	55.06b	53.46b	5.715	0.018 (*)
	Spd (cm)	58.48a	57.32a	56.63a	0.133	0.877 (ns)
Calamagrostis brachytrica	SDW (g)	8.89a	10.05a	8.18a	1.763	0.213 (ns)
	RDW (g)	27.76ab	29.41b	21.11a	3.66	0.057 (ns)
	Ht (cm)	61.94a	56.36a	55.44a	3.065	0.115 (ns)
	Spd (cm)	58.55a	58.29a	51.55a	1.476	0.267 (ns)
Caltha palustris	SDW (g)	3.66a	3.15a	3.19a	1.08	0.370 (ns)
	RDW (g)	10.44a	9.75a	8.51a	0.687	0.522 (ns)
	Ht (cm)	14.20a	14.04a	15.18a	0.235	0.794 (ns)
	Spd (cm)	26.32a	24.51a	22.89a	1.579	0.246 (ns)
Deschampsia flexuosa	SDW (g)	12.34a	13.02a	14.12a	0.371	0.702 (ns)
	RDW (g)	3.24a	2.57a	3.71a	1.55	0.278 (ns)
	Ht (cm)	65.86a	64.78a	64.54a	0.722	0.506 (ns)
	Spd (cm)	64.41a	62.02a	63.40a	0.509	0.614 (ns)
Filipendula purpurea	SDW (g)	14.09a	15.18a	16.93a	4.13	0.065 (ns)
	RDW (g)	44.64a	53.49a	41.91a	1.667	0.230 (ns)
	Ht (cm)	37.32a	40.42ab	47.80b	3.094	0.082 (ns)
	Spd (cm)	39.34a	38.74a	41.41a	1.342	0.298 (ns)
Gaura lindheimeri	SDW (g)	11.43a	12.12a	12.13a	0.329	0.724 (ns)
	RDW (g)	8.72b	7.32ab	6.35a	4.439	0.036 (*)
	Ht (cm)	94.38a	81.08a	81.82a	1.267	0.317 (ns)
	Spd (cm)	29.41b	24.85ab	21.94a	6.934	0.010 (**)
Hemerocallis 'Golden Chimes'	SDW (g)	14.91a	16.42a	16.01a	0.388	0.686 (ns)
	RDW (g)	19.96a	21.91a	16.64a	0.945	0.416 (ns)
	Ht (cm)	71.78a	70.16a	74.2a	0.338	0.720 (ns)
	Spd (cm)	50.64a	50.30a	46.78a	0.514	0.611 (ns)
Iris sibirica	SDW (g)	13.20a	16.60b	14.39ab	3.068	0.084 (ns)
	RDW (g)	26.64a	29.78a	24.30a	0.467	0.638 (ns)
	Ht (cm)	71.86a	74.78a	77.26a	1.539	0.254 (ns)
	Spd (cm)	38.55a	39.99a	42.34a	0.461	0.641 (ns)

Miscanthus sinensis	**SDW (g)**	14.00a	18.41a	18.05a	0.76	0.489 (ns)
	RDW (g)	34.44a	39.16a	32.59a	0.703	0.514 (ns)
	Ht (cm)	83.60a	100.36b	93.68ab	4.8	0.029(*)
	Spd (cm)	41.96a	82.18b	83.50b	29.658	<0.001 (***)
Molinia caerulea	**SDW (g)**	2.26a	4.54b	3.55ab	7.491	0.008 (**)
	RDW (g)	2.03a	4.19b	2.72ab	11.184	0.011 (*)
	Ht (cm)	36.92a	45.10a	42.16a	2.296	0.143 (ns)
	Spd (cm)	13.40a	29.70b	28.90b	31.742	<0.001 (***)
Rudbeckia fulgida var. deamii	**SDW (g)**	12.78a	11.66a	11.82a	0.657	0.536 (ns)
	RDW (g)	10.77a	9.94a	9.39a	1.097	0.365 (ns)
	Ht (cm)	34.44a	38.54a	34.84a	2.535	0.121 (ns)
	Spd (cm)	28.13b	25.33ab	23.82a	7.661	0.007 (**)
***Sanguisorba tenuifolia* 'Purpurea'**	**SDW (g)**	9.83b	8.95a	8.30a	0.704	0.514 (ns)
	RDW (g)	10.84b	8.43a	7.93a	4.587	0.033 (*)
	Ht (cm)	35.70a	41.54a	34.68a	1.104	0.363 (ns)
	Spd (cm)	37.55a	35.37a	34.48a	1.509	0.260 (ns)
Thalictrum aquilegifolium	**SDW (g)**	3.60a	3.42a	3.03a	0.52	0.616 (ns)
	RDW (g)	5.17a	5.14a	3.17a	2.658	0.111 (ns)
	Ht (cm)	35.76a	37.82a	31.72a	0.376	0.699 (ns)
	Spd (cm)	25.35a	21.81a	23.84a	1.33	0.301 (ns)
Veronicastrum virginicum	**SDW (g)**	10.81a	11.00a	10.95a	0.026	0.975 (ns)
	RDW (g)	16.61a	18.27a	16.78a	0.468	0.637 (ns)
	Ht (cm)	103.48a	97.04a	83.42a	1.818	0.204 (ns)
	Spd (cm)	24.10a	25.33a	25.30a	0.101	0.905 (ns)

Lowercase letters denote mean separation within columns; means with the same letter do not differ significant from each other.

ns = not significant, *=between 0.05 and 0.01 **= between 0.01 and 0.001 and ***=<0.001

At least one of the measurable growth parameters in those of other selected species was significantly affected by the cyclic flooding treatments. *Astilbe 'Purple Lance', Miscanthus sinensis* and *Molinia caerulea* showed increases in growth due to the treatments of cyclic flooding. *Molinia caerulea* showed significantly increased SDW and RDW in 1d cyclic flooding treatment compared with the control group, whilst the two growth characteristics in this species obtained from 4d group were not statistically different from those of 1d group and the control group. *Astilbe* 'Purple Lance' and *Miscanthus sinensis* showed least height growth in the control group, while mean height value in the two species from 1d and 4d group was not independent from each other. Significantly increased canopy spread in *Miscanthus sinensis* and *Molinia caerulea* was determined in both 1d and 4d group compared with the regularly irrigated control group, whilst no statistical difference was determined between the mean spread values in 1d and 4d group.

The other species affected by the treatments of flooding cycles showed significant reduction in some of the growth characteristics due to the longer-term (4d) cyclic flooding treatment, while tolerances to 1d cyclic flooding were indicated. 4-day interval cyclic submergences significantly reduced RDW in *Amsonia tabernaemontana var. salicifolia, Gaura lindheimeri,* and *Sanguisorba tenuifolia* 'Purpurea'. Mean RDW values in the three species obtained from 1d group and the control group were not independent from each other. Significant canopy spread reduction in *Gaura lindheimeri* and *Rudbeckia fulgida var. deamii* was determined in 4d group compared with the control group, while no statistical difference was determined between the mean spread values in 1d group and the control group.

Measurements of leaf chlorophyll fluorescence (F_v/F_m) in individual species are shown in

Fig. 3. Due to technical issues, it was not possible to obtain F_v/F_m ratio in *Deschampsia flexuosa*. Interpretations of what the results mean are made for each species.

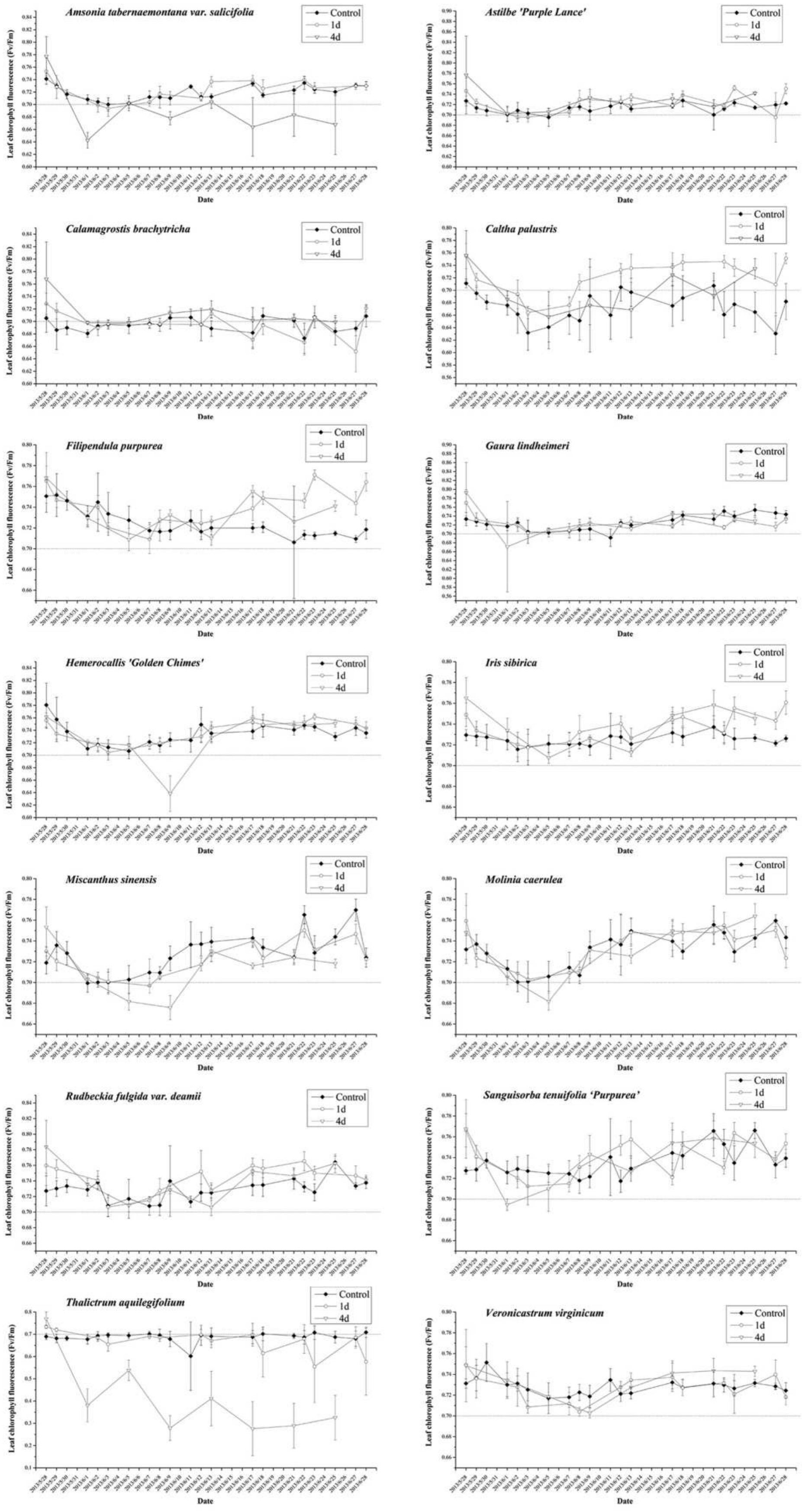

Fig. 3. Changes in the mean values of chlorophyll fluorescence from the control, 1d and 4d treatments in individual species. Error bars represent standard error.

The time series F_v/F_m from all treatments for *Filipendula purpurea*, *Iris sibirica*, *Rudbeckia fulgida var. deamii* and *Veronicastrum virginicum* was consistently above 0.7 throughout the whole study, which indicates the best stress tolerance to cyclic flooding among all selected species. Such results generally matched with their growth characteristics that no significant effects of cyclic flooding were indicated. A recovery trend for F_v/F_m in *Filipendula purpurea* and *Iris sibirica* was found during flooding periods, whereas F_v/F_m recovery in *Rudbeckia fulgida var. deamii* and *Veronicastrum virginicum* was determined during draining stages.

F_v/F_m profile in *Gaura lindheimeri, Hemerocallis* 'Golden Chimes', *Molinia caerulea* and *Sanguisorba tenuifolia* 'Purpurea' only occasionally fell below 0.7 in 4d cyclic flooding group, but the overall performances were positive. *Molinia caerulea* and *Sanguisorba tenuifolia 'Purpurea'* showed increasing chlorophyll fluorescence during flooding stages, whereas recovering F_v/F_m during draining stages was determined in *Gaura lindheimeri* and *Hemerocallis* 'Golden Chimes'. The general performances of the four species matched with their growth characteristics. A moderate tolerance to cyclic flooding stress was exhibited by *Gaura lindheimeri*, and this did not match with its growth performance, in which significant reduction of root dry weight and canopy spread was determined due to the longer-term (4d) cyclic flooding treatment.

Flooding-induced stress (i.e. $F_v/F_m < 0.7$) was occasionally determined in *Astilbe* 'Purple Lance' and *Miscanthus sinensis* in both 1d and 4d group during the draining stages. Considering the fact that height of the two species and the spread of *Miscanthus sinensis* were increased due to cyclic flooding treatments, their vigorousness was therefore determined.

Poor stress tolerances were exhibited by *Calamagrostis brachytrica* and *Thalictrum aquilegifolium* from both 1d and 4d group and *Amsonia tabernaemontana var. salicifolia* and *Caltha palustris* from 4d group, in which more than 50% of the total F_v/F_m measurements were detected lower than 0.7. *Amsonia tabernaemontana var. salicifolia* and *Thalictrum aquilegifolium* could only recover their photosynthesis efficiency during the draining stages. Obvious leaf necrosis showed in *Thalictrum aquilegifolium* in 4d group during the third flooded treatment, which indicated extreme plant stress. Such performances in *Amsonia tabernaemontana var. salicifolia* and *Thalictrum aquilegifolium* did not match with their physical growths that no significant flooding-induced reducation in growth characteristics were determined. *Calamagrostis brachytrica* and *Caltha palustris* showed stress due to the shortages of soil moisture during the draining stages, and only recovered chlorophyll fluorescence in waterlogged or damp soils.

Scores of survival, physical growths and stress tolerance in each species are presented in Table 3. Friedman test was applied on these ordinal-scale data, which indicates that significant differences between species using the ratings ($P = 0.004$). A league table base on mean rank is thus presented to show the level of suitability across different species in cyclic flooding treatments (Table 4).

Table 3. Summary scores of cyclic flooding performances in individual species, including survival, shoot dry weight (SDW), root dry weight (RDW), height (Ht), spread (Spd) and stress tolerance

Species	Survival	SDW	RDW	Ht	Spd	Stress tolerance
Amsonia tabernaemontana var. salicifolia	5	4	2	4	4	2
***Astilbe* 'Purple Lance'**	5	4	4	6	4	3
Calamagrostis brachytrica	5	4	3	3	4	1
Caltha palustris	5	4	4	4	4	2
Deschampsia flexuosa	5	4	4	4	4	/[a]
Filipendula purpurea	5	5	4	5	4	5
Gaura lindheimeri	5	4	2	4	1	4
***Hemerocallis* 'Golden Chimes'**	5	4	4	4	4	4
Iris sibirica	5	5	4	4	4	5
Miscanthus sinensis	5	4	4	6	7	3
Molinia caerulea	5	7	6	5	7	4
Rudbeckia fulgida var. deamii	5	4	4	5	1	5
***Sanguisorba tenuifolia* 'Purpurea'**	5	4	2	4	4	4
Thalictrum aquilegifolium	5	4	3	4	4	1
Veronicastrum virginicum	5	4	4	4	4	5

a: F_v/F_m was not obtained in *Deschampsia flexuosa* due to technical issues.

Table 4. League table based on mean rank of individual species' performance in cyclic flooding treatments. Higher-scored species showed better suitability and overall performance.

Species	Mean Rank
Calamagrostis brachytrica	4.92
Gaura lindheimeri	5.5
Amsonia tabernaemontana var. salicifolia	5.67
Thalictrum aquilegifolium	5.75
***Sanguisorba tenuifolia* 'Purpurea'**	6.58
Caltha palustris	7
Deschampsia flexuosa	7.92
***Hemerocallis* 'Golden Chimes'**	7.92
Rudbeckia fulgida var. deamii	8.58
Veronicastrum virginicum	8.67
***Astilbe* 'Purple Lance'**	8.75
Iris sibirica	9.83
Miscanthus sinensis	9.83
Filipendula purpurea	10.83
Molinia caerulea	12.25

4 Discussion

Although there was 100% survival in all 15 species during the cyclic flooding treatments the degree of growth and physiological response varied. As stated previously, most established technical guidance suggests proper engineering for rain gardens to achieve complete dewatering within 24 hours. All the 15 candidate perennial species showed suitability to this ideal rain garden moisture regime because no species had significantly decreased growth characteristics due to the 1d interval cyclic flooding. We therefore strongly recommend suitable soil engineering in situ to enhance water discharge, so that a wider range of potential species could be considered for use in urban rain gardens.

The present experiment only applied submergences to substrate level. We therefore assumed that root growth is the most sensitive growth characteristic due to the possible damages on root metabolism and nutrient acquisition caused by periodic hypoxia and anoxia resulting from cyclic submergences [29]. The assumption is proved by the investigation, where the longer-term (4d) interval cyclic flooding significantly decreased the root biomass in *Amsonia tabernaemontana var. salicifolia, Gaura lindheimeri,* and *Sanguisorba tenuifolia* ‘Purpurea’.

Canopy growths in most candidate species responded positively to the influence of cyclic flooding treatments, especially in the one-day short interval flooding cycles. Casanova and Brock [30] concluded similar results that short frequent floods promoted high biomass of two types: the amphibious species that established their tolerance to the fluctuated inundating-draining process, and those terrestrial species that are capable of growing fast and establishing themselves during the period of draining between floods.

In this study, leaf chlorophyll fluorescence as the indicator for evaluating cyclic flooding tolerance is able to reveal the invisible biological damages in the candidate plants to predict stress. This method is less destructive to plants and require less time to reveal stress in plants compared to the traditional means by measuring the physical growth of plants. In actual assessment, chlorophyll fluorescence provides additional and novel insights into species’ tolerance to flooding cycles. Most species maintained relatively good F_v/F_m level during the whole study, which matched their overall physiological growth conditions, and thus demonstrated their adaptative responses to the experimental cyclic flooding. *Gaura lindheimeri* showed significantly decreased root dry weight and canopy spread in treatments adopted 4d cyclic flooding, whilst this species established stress tolerance to both 1-day and 4-day cyclic flooding. It indicates that *Gaura lindheimeri* could be a waterlogging avoider postpones growth to thrive in flood-prone environments. *Amsonia tabernaemontana var. salicifolia* in 4d group and *Thalictrum aquilegifolium* in both 1d and 4d cyclic flooding treatments showed poor health conditions (i.e. more than half of the F_v/F_m measurements were found below 0.7) with rather limited recovery of photosynthetic efficiency during draining stages. We assume potential biological injury might occur or become visibly apparent in these plants if more flooding cycles were provided. The assumption was supported by the fact that leaf necrosis occurred in *Thalictrum aquilegifolium* during the third 4-day flooding treatment.

Statistical analysis based on candidate species’ independently scored performances in survival, physical growths, as well as chlorophyll fluorescence suggest significant between-species differences in their resilience to cyclic flooding treatments. *Iris sibirica*, *Filipendula purpurea*, *Miscanthus sinensis* and *Molinia caerulea* are the highest scored among the 15 candidate species, whilst *Astilbe* 'Purple Lance', *Deschampsia flexuosa*, *Hemerocallis* 'Golden Chimes', *Rudbeckia fulgida var. deamii*, *Sanguisorba tenuifolia* ‘Purpurea’ and *Veronicastrum virginicum* also showed adaptive responses to simulated rain garden cyclic flooding. Most of these species are therefore considered suitable for all the three rain garden saturation zones (i.e. margin, slope and bottom) in a wide range of climate conditions. It is noticeable that F_v/F_m profile showed that photochemical efficiency recovery in *Astilbe* 'Purple Lance', *Iris sibirica*, *Filipendula purpurea* and S*anguisorba tenuifolia* ‘Purpurea’ generally occurred in flooding periods, and are therefore considered suitable for rain gardens in regions with greater annual rainfall volume. Poor tolerance to rain garden cyclic flooding was determined in *Amsonia tabernaemontana var. salicifolia*, *Gaura lindheimeri* and *Thalictrum aquilegifolium*. The three species are not preferred for longer internal cyclic flooding, and thus should neither be adopted in the frequently damp depression bottoms of rain gardens, nor the slopes with poorly-drained soils in a humid climate. *Calamagrostis brachytrica* and *Caltha palustris* scored rather low among all the candidate species, which is largely due to their poor stress tolerance showed in the control group, whilst tolerance in the two species was built through the increasing number of flooding cycles. The two species could therefore be adopted at the basin bottom in a poorly drained soil. Table 5 shows which of the three rain garden saturation zones each species is best fitted for, according to the results.

Table 5. Suggestion of species distribution in different saturation zones and preconceived assumptions about the moisture sensitivity of each species

Species	Margin	Slope	Bottom	Assumed moisture sensitivity
Amsonia tabernaemontana var. salicifolia	●	●		Infrequent inundation
***Astilbe* 'Purple Lance'**	●	●	●	Periodic or seasonal inundation
Calamagrostis brachytrica			●	Periodic or seasonal inundation
Caltha palustris			●	Continuous inundation
Deschampsia flexuosa	●	●	●	Periodic or seasonal inundation
Filipendula purpurea	●	●	●	Periodic or seasonal inundation
Gaura lindheimeri	●	●		Intolerant of inundation
***Hemerocallis* 'Golden Chimes'**	●	●	●	Infrequent inundation
Iris sibirica	●	●	●	Infrequent inundation
Miscanthus sinensis	●	●	●	Periodic or seasonal inundation
Molinia caerulea	●	●	●	Periodic or seasonal inundation
Rudbeckia fulgida var. deamii	●	●		Infrequent inundation
***Sanguisorba tenuifolia* 'Purpurea'**	●	●	●	Infrequent inundation
Thalictrum aquilegifolium	●			Infrequent inundation
Veronicastrum virginicum	●	●	●	Periodic or seasonal inundation

●: Possible placing of species in different plant moisture zones

Assumptions of selected species' moisture sensitivity to different hydrological regimes is also presented in Table 5, which is often used as a basis for proposing suitable plant species in established rain garden guides. Hydrological regime can be described by the duration, frequency, timing and predictability of the flooded and dry phases [31]. Assumed moisture sensitivities of plants often tend to be determined according to their tolerance to fluctuation in flooding and drying documented in a variety of botanic guides for gardeners [2, 7, 22, 23, 32], and may often be correctly predicted depending on the habitats where they are found in nature. In general, four levels of moisture sensitivities are recognised, which range from: (1) continuous inundation (i.e. 'wetland' species), (2) periodic or seasonal inundation (i.e. species from wet meadows or other habitats that are not permanently wet), (3) infrequent inundation (i.e. species from fertile habitats in temperate maritime climates), and (4) intolerant of inundation (i.e. species from dry or arid habitats). Most of the given species in this study were assumed to withstand the periodic/ seasonal inundation or to withstand infrequent inundation, which are the two most popular options in established guidances. *Caltha palustris* was indigenous in regular saturated conditions, and *Gaura lindheimeri* was assumed to be intolerant of inundation. These two species were chosen to represent the potential extremes of condition in a rain garden context.

The recognised moisture sensitivities to different hydrological regimes and the original wild habitats in each species match the preconceived assumptions about which rain garden zone each species is best fitted to. Perennials are established in planting positions appropriate to their ecological needs, resulting in greater longevity and lower maintenance demands [23]. We consider the methodology adopted in this study is confident in predicting the potential of the species being tested for rain garden use, and allow practitioners predicting the suitability for different zones in a rain garden for any candidate species. In the current study, species assumed to withstand infrequent and periodic inundation, especially those of naturally growing in transition zone between upland and wetland (e.g. moist meadows and swamps), showed the best performances over the species inhabitating the other hydrological regimes. We consider these species are sensible to use for all saturation zones ranging from the damp depression bottom to the relatively dry marginal area, which may therefore gain popularity in future applications. For instance, the highest scored *Iris sibirica* is naturally found in swamps and damp pastures, while *Filipendula purpurea* and *Miscanthus sinensis* naturally grow alongside stream margins or moist lowland meadows where periodic inundation occurs at time.

In this study, the basic 'pot-in-pot' methodology sccuessfully simulated interval cyclic flooding conditions occurring in rain gardens. However, it is undoubtedly that the use of container-grown plants would have influence to the experimental observations. Considering the potentially high transpirational water loss due to the elevated temperature in greenhouse during the study and the free-draining medium with limited volume in pots, availability of soil moisture in pot is expected to rapidly decrease during the draining stages and thus challenge the planting success of some of the moisture-needy species. Such risks may be weaker in practical rain garden as more soil moisture is expected to be maintained in planting beds and soils at different depths.

Greater volume of organic component were adopted in the growing medium of the work of Bailey [24] and Dylewski *et al*. [12] compared to that we used in this study. For instance, Bailey used a 9:1 pine bark: sand by volume medium and Dylewski et al. adopted 1:1 pine bark: peat by volume medium, whereas we used a sandy textured medium in which half volume was sharp sands. The volumetric water content determined greatly by organic component in the medium, which means the daily-maintained substrate per cent moisture between 20% and 25% in the control group in this present study may lose moisture easier than that of Bailey and Dylewski *et al*. and might not be able to maintain a mesic substrate. It might help explain why often control plants did not grow as well as the 1-day flooding, especially in the species showing preferences for greater moisture levels.

5 Conclusion

Plant health plays a major role in maintaining the functionality and aesthetics of rain gardens, therefore rain garden successes dependent on proper species choice. This study represents a step in adapting the measurements of plant growth characteristics such as SDW, RDW, height and spread coupled with the stress indicator (i.e. chlorophyll fluorescence) to identify tolerant species and ecotypes for the typical cyclic flooding scenarios in rain gardens. This study is thus valuable for guiding future collaborative research and application to choose species that are likely to be suited to life in differing soil moisture conditions throughout the depression structure of rain garden.

This experiment terminated in one month as the tested perennials in the control, 1d and 4d group reached their maximum in greenhouse with an elevated temperature. As stated previously, a few candidate species may show differing stress conditions if more flooding cycles were given. The extreme indoor temperature is considered a potential limitation, so that the future research is recommended to be carried out under a stable range of temperature. However, species could become increasingly tolerant of flooding as plants mature [33], thus results of most species are valuable for predicting their further adaptions to cyclic flooding treatments.

In practical rain garden conditions, plants may experience weather shocks such as moving rapidly from drought to flood or the reverse. It is valuable to design a controlled condition with a repetitive cycle that rapidly switches between extremely low soil moisture and inundation to know how the plants cope with weather shocks and to identify suitable species for extreme conditions. Additionally, plants growing in rain garden bottoms may occasionally encounter deeper flooding to leaf level, and cause direct shading and hypoxia to foliage. It is also valuable to know the interaction between plant establishment and periodical deeper inundation in future research. This was not done due to significant loss of substrates from pots in deeper inundation over substrate level, and is thus expected to be investigated in practical rain gardens.

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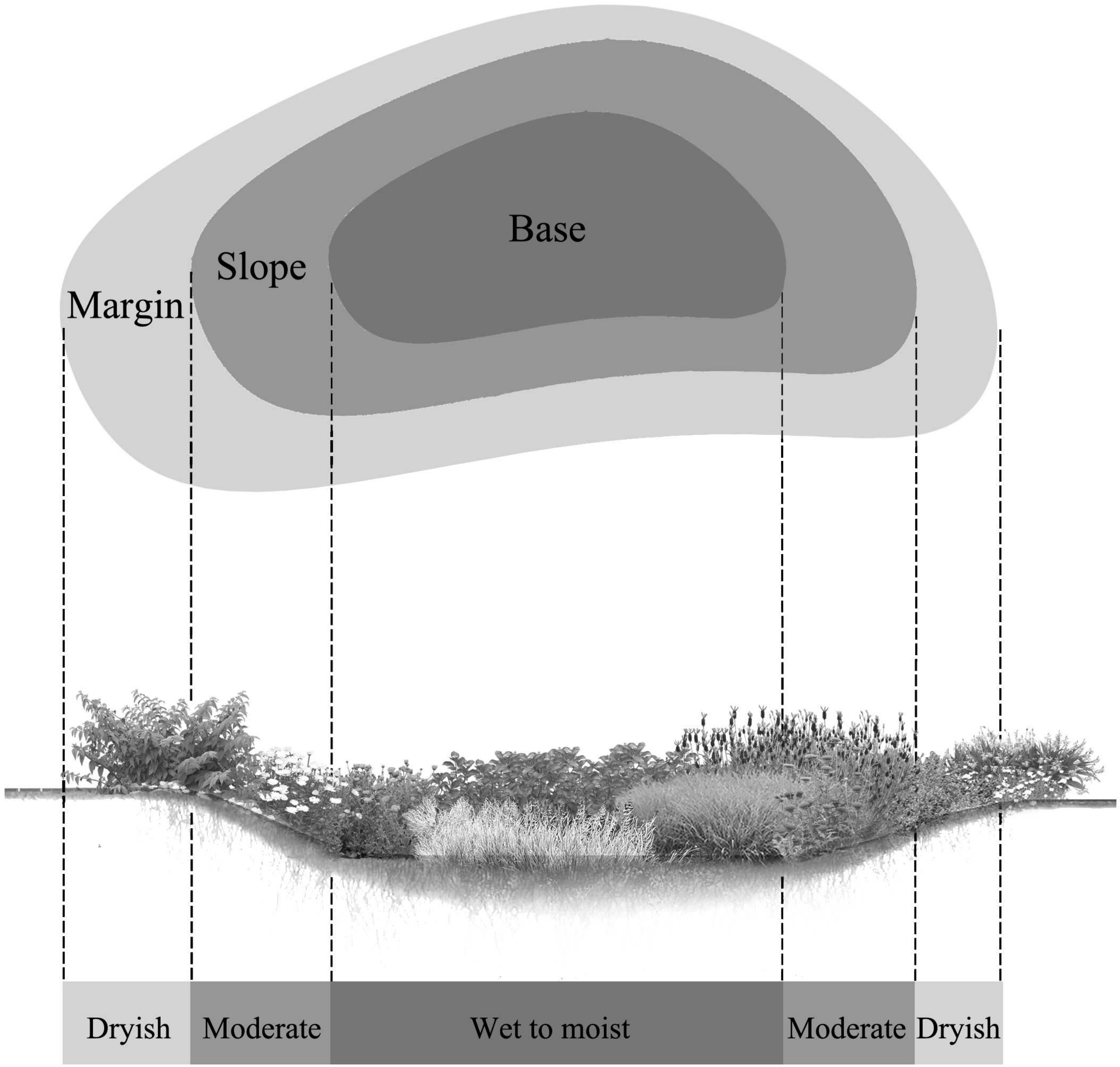
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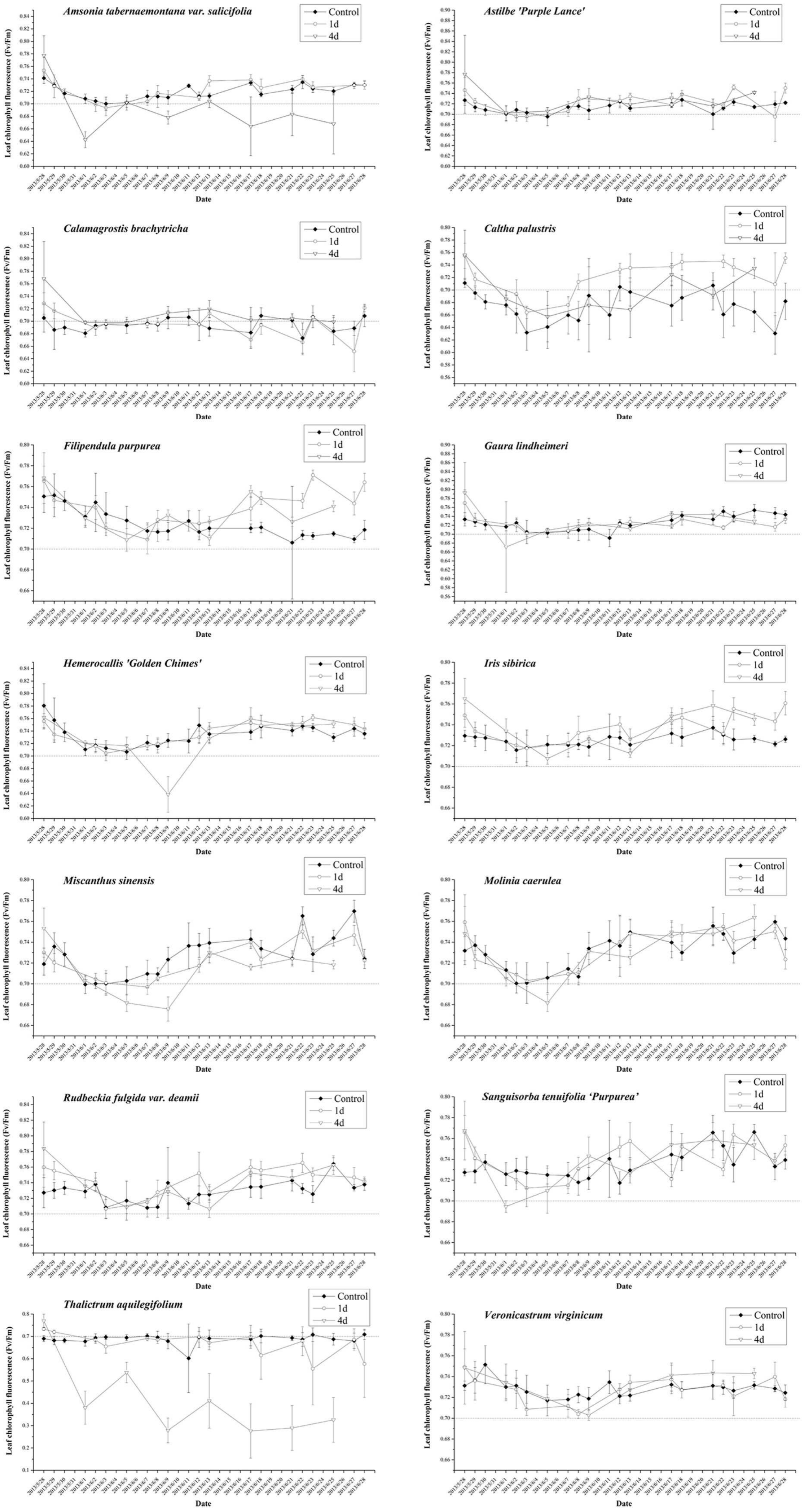

Amsonia tabernaemontana var. salicifolia
Astilbe 'Purple Lance'
Calamagrostis brachytricha
Caltha palustris
Filipendula purpurea
Gaura lindheimeri
Hemerocallis 'Golden Chimes'
Iris sibirica
Miscanthus sinensis
Molinia caerulea
Rudbeckia fulgida var. deamii
Sanguisorba tenuifolia 'Purpurea'
Thalictrum aquilegifolium
Veronicastrum virginicum
Control
1d
4d
Leaf chlorophyll fluorescence (Fv/Fm)
Date